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Use of n-butanol for efficient recovery of minute amounts of small RNA fragments and branched nucleotides from dilute solutions

Guy Cathala and Claude Brunel
URA CNRS 1911, Laboratoire de Biochimie, Centre Paul Lamarque, 34094 Montpellier Cedex 2 and Laboratoire de Biologie Moléculaire USTL, 34095 Montpellier Cedex 2, France

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n-butanol extraction to concentrate dilute aqueous solutions of nucleic acids has been known for a long time but used exclusively for DNA. We describe here two situations in which n-butanol extraction proved particularly useful and efficient in the case of RNA.

1) Recovery of small RNA fragments from urea-SDS polyacrylamide gels.

Gel slices corresponding to small as RNase T1 fragments are eluted overnight in an Eppendorff tube in 300 μl of 0.7 M NH^4Ac, 1mM EDTA, 0.1% SDS and 10 μg carrier tRNA if necessary. The supernatant freed of acrylamide debris by a short spin is transferred into another tube which is then filled with n-butanol, vigorously shaken for 30 sec and centrifuged for 1 min. The upper organic phase is discarded and the aqueous phase is exhausted by repeated extractions until RNA appears as a pellet which is then collected by a 10 min centrifugation. The pellet is redissolved in 200 μl of water and dried by a single n-butanol extraction. After three such cycles followed by vacuum drying, the RNA pellet is ready for use. This protocol completely removes salts, SDS and urea. It is simpler and faster than any other. It has no lower size limit, except for mononucleotides, since it is not a precipitation. Its yield is quantitative and does not depend on the amount of material. As an example, a small RNA fragment (even < 10 nt) containing 50 cpm (cerenkov) is easily recovered to be further submitted to RNase digestions and analysed by thin layer chromatography. This procedure applies equally well to DNA fragments (for example synthetic oligonucleotides).

2) Characterization of branched nucleotides.

n-butanol extraction turned out to be also invaluable to characterize the very small amount of branched nucleotide generated during pre-mRNA splicing. In this case, mononucleotides resulting from alkaline hydrolysis of a total splicing reaction mixture are removed in the order A>U>C>G as expected from the hydrophobicity of their bases, so that the alkaline hydrolysis resistant branched trinucleotide appears as a major species in thin layer chromatography (Figure 1). In fact, the extraction procedure was purposely not exhaustive so as to keep enough mononucleotides as markers.

Solvents were isobutyric acid concentrated NH_4OH-H_2O (66/1/33, v/v/v) in the first dimension and 0.1 M sodium phosphate, pH 6.8-ammonium sulfate-n-propanol (100/60/2, v/w/v) in the second dimension.

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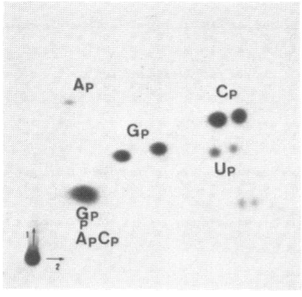

Figure 1. Analysis of in vitro RNA processing products by alkaline hydrolysis and thin layer chromatography. RNA processing products from 10^6 cpm of β-globin pre-mRNA labeled with ^{32}P UTP were generated during 1 hr. under standard conditions (1), phenol-chloroform extracted and ethanol precipitated to remove glycerol and polyvinyl alcohol and then submitted to alkaline hydrolysis (2). After neutralization and centrifugation, the mixture was extracted three times by n-butanol as described above in the presence of 10 μg tRNA carrier, and analyzed by thin layer chromatography using cellulose plates (3).